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CONTENT OF AMINO ACIDS, FATTY ACIDS AND SOME GLYCIDES IN THE FUNGUS *STACHYBOTRYS ALTERNANS*

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In a study of the biochemical properties of the fungus *Stachybotrys alternans*, whose toxins are the cause of a mycotoxicosis of cattle and horses (Sarkisov, 1954; Forgacs and Carll, 1962; Spesivtseva, 1964; Beseda et al., 1965), we considered it necessary first to acquaint ourselves with the basic materials which make up its cell structure. In this article we present the composition of the amino acids, the fatty acids, and the sugars as we determined it in hydrolysates of the mycelium of the species.

Material and Methods

Cultures of *Stachybotrys alternans* after two weeks' incubation at 25°C on Czapek-Dox agar of the usual composition were carefully scraped, without damaging the agar layer, and hydrolyzed 18 hours at 105°C in fused ampoules, some with 6 N HCl and some with 1 N H_2SO_4. The hydrolysate with hydrochloric acid was used for determining the amino acids and the hydrolysate with sulfuric acid for determining the glycides. The determination of the amino acids and sugars was done by chromatography and by high-voltage paper electrophoresis (Ferenčík, 1962 a,b; Ferenčík and Čižnár, 1965).

The lipoids were extracted from dried cultures of the fungus with a mixture of chloroform and methyl alcohol (2:1). After evaporation of the extract in an atmosphere of nitrogen, a 2 N methyl alcohol solution of KOH was added to the residue and the mixture hydrolyzed for 6 hours on boiling water and then cooled. After cooling, the hydrolysate was diluted with water, acidulated with hydrochloric acid to pH 3.0, and extracted with ether. After evaporation of the ether the residue was dissolved in an equal quantity of chloroform, which was transferred to Whatman 3 paper impregnated with paraffin oil. The various fatty acids were separated

chromatographically by the use of 93% acetic acid as a mobile phase. The same procedure has been described in another place (Krajčiová et al., 1962).

Besides *S. alternans*, for the sake of comparison we also analyzed several other fungi. All these fungi were isolated in feed for domestic animals.

The toxicity of the individual strains was tested on the skin of the rabbit, on the species *Tetrahymena pyriformis*, and by Vasin's method (Ladzianska, 1967).

Results and Discussion

All the fungi studied contained 18 amino acids: cystine, lycine, histadine, arginine, glycine, serine, threonine, alanine, proline, tyrosine, methionine, valine, phenyl-alanine, leucine, isoleucine, tryptophan, and aspartic and glutamic acids. In the mycelium of some fungi there were also other amino acids, namely α-aminobutyric acid (ABA), citruline (Cit), ornithine (Orn), and taurine (Tau) (Table I).

Table I. Occurrence of some amino acids.

Fungus	Toxicity	Origin	α-ABA	Cit	Orn	Tau
S. alternans 14 and 857	++++	straw	+	+	+	-
S. alternans 36 and 93	++++	straw	-	-	+	-
S. alternans 862	++++	straw	-	-	-	+
S. alternans 1282	++++	wall scrapings	-	-	-	+
S. alternans 276 & 1249	+++	straw	-	-	-	-
S. alternans 19	+++	silage	-	-	-	-
S. alternans 815	-	wall scrapings	-	-	-	-
A. niger	-	mixed feed	-	+	+	+
A. fumigatus	-	mixed feed	-	+	+	+
A. flavus	-	mixed feed	-	+	+	+
A. clavatus	-	mixed feed	-	+	+	+
Fusarium sp. 1 and 3	-	hay	-	+	+	+
Fusarium sp. 2	-	hay	-	+	+	-

It is interesting that the four amino acids mentioned are found in *S. alternans* only in the strains of high toxicity. The mycelium of the comparison types *Aspergillus* and *Fusarium* contained Cit, Orn, and Tau, but not α-ABA.

Strains 14 and 857 of *S. alternans* we analyzed after the first, fifth, eleventh, twentieth, and twenty-ninth passages through solid Czapek-Dox medium. After passages 1 and 5 both strains contained Cit and Orn; after the eleventh and later passages we no longer found

Table II. Occurrence of some glycides.

Fungus	MAL	MAN	XYL	RAM	GLU-UA	GAL-NH_2
S. alternans 14	+	+	+	+	+	-
S. alternans 857	+	+	-	-	+	-
S. alternans 93	-	-	+	-	+	-
S. alternans 276	-	+	+	-	-	-
S. alternans 815	-	+	+	-	+	-
A. niger	-	+	±	-	-	-
A. fumigatus	-	+	±	-	-	-
A. flavus	-	+	±	-	-	+
Fusarium sp. 1 and 3	-	+	±	-	-	+
Fusarium sp. 2	-	+	±	-	-	-

Table III. Occurrence of some fatty acids.

Fungus	ARA	OLE	LIN	"X"
S. alternans 14	+	+	-	+
S. alternans 857 and 1282	-	+	-	+
S. alternans 862	+	+	+	-
S. alternans 93	-	+	+	-
S. alternans 276	±	+	-	-
A. niger	-	+	+	-
A. fumigatus	-	+	+	-
A. flavus	-	-	+	-
Fusarium 1 and 2	+	+	+	-

these two amino acids in them, but a new amino acid, namely taurine, did show up. This fact may be connected with certain metabolic changes, chiefly in the urea cycle.

Of the sugars all the fungi examined showed galactose, glucose, glucosamine, arabinose, and raffinose. We found certain differences with respect to maltose (MAL), mannose (MAN), xylose (XYL), glucuronic acid (GLU-UA), and galactosamine (GAL-NH_2) (Table II).

Some strains of *S. alternans* contained MAL, MAN, XYL, GLU-UA, and one of them ramose (RAM). All the strains of *Aspergillus* and *Fusarium mali* contained MAN and traces of XYL, and some of them GAL-NH_2 as well. We found no changes in the sugar composition analogous to those found in

the amino acids with passages of *S. alternans* through the Czapek-Dox medium.

Of the saturated fatty acids we found stearic, palmitic, and myristic acid in all the strains of fungi, and of the unsaturated fatty acids linoleic acid. The mycelium of some strains of *S. alternans* contained arachidic (ARA), oleic (OLE), and linolenic (LIN) acids in addition, together with an unidentified one ("X"). This last was found in only one case among the other fungi (Table III).

Arachidic acid was found in the strain *S. alternans* only in the first passage. In the strain *S. alternans* 857 oleic and linoleic acids were found only after the fifth and later passages through Czapek-Dox solid medium. The latter strain contained in addition to these two the unidentified fatty acid, which was also present in strains 14 and 1282.

All the results of analysis shown in Tables I to III were found in the fungi after the third or fourth passage through solid Czapek-Dox medium.

The observed changes in the composition of the amino acids and fatty acids which came about in the course of long passages (29 passages) through Czapek-Dox medium in certain strains of *S. alternans* are evidently caused by variations in the composition of the substrates which the fungi had at their disposal as compared to the natural environment from which they were isolated. The toxicity of the strains investigated was practically unchanged in this series of passages, and the change in the amino acid and fatty acid content had no probable connection with the production of the toxins.

Summary

The authors determined the amino acid, fatty acid, and glycide content of the mycelium of *Stachybotrys alternans*, and, for purposes of comparison, of some strains of *Aspergillus* and *Fusarium* as well. The hydrolysates contained 18 to 22 various amino acids, 5 to 11 sugars, 3 to 4 saturated and 1 to 5 unsaturated fatty acids. In the case of *Stachybotrys alternans* the individual strains exhibited small differences in the composition with respect to these substances. Differences in the occurrence of amino acids and fatty acids were found in some strains of *Stachybotrys alternans* even after long-lasting passages through solid Czapek-Dox substrate. These changes, however, were not related to toxin production. ( )

BIBLIOGRAPHY

1. Beseda, M., Ladzianska, K., and Halík, J., *Mykotoxikózy Hospodárskych Zvierat* (Mycotoxicosis of Domestic Animals), Slov. Vyd. Pôdohosp. Lit. (Slovak Agricultural Press), Bratislava, 1965.

2. Ferenčík, M., "Determination of Histamines and Histidines by High-Voltage Paper Electrophoresis," *Chemické Listy* (Chemical Notes), Vol. 56, 1962, page 289.

3. Ferenčík, M., "Determination of Amino Acids and Amines by High-Voltage Electrophoresis on Papers with the Use of Damping Solutions of Low Ionic Strength," *Veterinární Časopis* (Veterinary Journal), Vol. 11, 1962, page 306.

4. Ferenčík, M. and Čižnár, I., "Separation of Sugars by High-Voltage Electrophoresis on Papers," *Chemické Listy*, Vol. 59, 1965, page 332.

5. Forgacs, J. and Carll, W.T., "Mycotoxicoses," *Advances in Veterinary Science*, Vol. 7, 1962, page 273.

6. Krajčiová, V., Čižnár, I., and Kreméry, V., "Chromatographic Determination of Fatty Acids in Endotoxins and in Whole Cells of *Salmonella cholerae suis*," *Biológia* (Biology), Vol. 17, Bratislava, 1962, page 148.

7. Ladz nska. K.. "Proof of the Toxicity or *Stachybotrys alternans*," *Veterinárná Medicína* (Veterinary Medicine), 1967, to be published.

8. Sarkisov, [illegible]., *Mikotoksikozy* (Mycotoxicoses), Gos.Izd.Sel'skokhoz.Lit. (State Agricultural Press), Moscow, 1954.

9. Spesivtseva, N.A., *Mikozy i Mikotoksikozy* (Micoses and Micotoxicoses), Moscow, 1964.